

Short Communication

Preparation and Use of an Autogenous Bacterin against Fowl Cholera in Red Partridges (*Alectoris graeca*)

K. BOUZOUBAA¹, B. HARIF¹, M. EL HOUADFI¹, M. OUCHEN², P. BERTIN² and A. GRINI³

¹Département de Pathologie Aviaire, Institut Agronomique et Vétérinaire Hassan II, BP 6202 Rabat-Instituts (Morocco)

²Société de Productions Biologiques et Pharmaceutiques Vétérinaire (BIOPHARMA), BP 4569 Rabat-Akkari (Morocco)

³Laboratoire de Recherches et d'Analyses des Services Vétérinaires, BP 4509 Rabat-Akkari (Morocco)

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ABSTRACT

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Pasteurella multocida serotype A₈ was isolated from red partridges (*Alectoris graeca*) in an outbreak of fowl cholera in Morocco. An autogenous bacterin was prepared and then used experimentally. Birds were vaccinated at 6 and 8 weeks of age and challenged intramuscularly 3 weeks after the second vaccination. The two aluminium hydroxide bacterins, prepared on different culture media (Trypticase soja agar and brain-heart infusion broth), conferred significant and similar immunity in partridges giving 60% protection. All unvaccinated controls died within 24 h.

INTRODUCTION

Fowl cholera caused by *Pasteurella multocida* is a contagious disease affecting domesticated and wild birds.

In June 1985, an outbreak of fowl cholera in 7-10-week-old red partridges (*Alectoris graeca*) was reported in Morocco (Bouzoubaa et al., 1987). *P. multocida* was isolated and identified as serotype A₈ (Carter, 1955; Heddelston et al., 1972). During the outbreak, a few drugs proved to be effective, but usually only for as long as treatment lasted. While antibiotics and chemotherapeutic substances such as long-acting terramycin and trimethoprim were helpful in treating sick birds, they were inadequate in eradicating the disease on the farm. The improved hygiene and management practices including wild-bird control

and the control of human movement were also not always sufficient to overcome losses due to the outbreak. No previous history of fowl cholera on the premises was reported. Autogenous vaccines have been reported to be effective in coping with fowl cholera (Michael et al., 1976).

Since the treatment was not totally satisfactory and in order to prevent further infection in future flocks, it was decided to use an autogenous vaccine with the inactivated *P. multocida* serotype A₈ isolate. The present paper describes the results of the production and the use of an autogenous bacterin to prevent fowl cholera in a flock of partridges in Morocco.

MATERIALS AND METHODS

Experimental birds

Laboratory trials were carried out in 5-week-old partridges of commercial origin. They were ascertained to be *P. multocida* - negative by culturing cloacal and tracheal swabs. They were then housed in isolated pens at the Institut Agronomique et Vétérinaire Hassan II in Rabat, Morocco. Usual poultry facilities (food and water supply, appropriate disinfection measures taken after each visit) were provided for their care and management.

Feed

Commercial feed containing no antibiotics was used. Clean water was provided ad libitum. Samples of feed were cultured bacteriologically to confirm that feed was negative for *P. multocida* and for *Salmonella*.

Bacteria

The strain of *P. multocida* used was isolated from red partridges in Morocco during an outbreak of fowl cholera. A diagnosis based on serotyping by passive haemagglutination (Carter, 1955) and gel-diffusion precipitating test (Heddelston et al., 1972) identified the isolate as serotype A₈ *P. multocida*. The organism was designated PMM8.

Bacterin preparation

Two bacterins were prepared from a broth culture and on solid medium.

The lyophilized strain of PMM8 was reconstituted in trypticase soja broth and then streaked on a blood agar plate. The plate was incubated for 24 h at 37°C, and a single smooth iridescent colony showing encapsulated organisms (Jasmin, 1945) was transferred to 35 ml of brain-heart infusion broth (BHB) and incubated for 24 h. Three millilitres of this broth culture were seeded on

trypticase soja agar in Roux bottles and incubated at 37°C for 48 h, and 15 ml of the same broth culture were used to inoculate 3 l of BHB and incubated for 30 h at 37°C. The growth on Roux bottles was washed off in 50 ml of saline. Sterile pointers were used to remove the cells from the surface of the agar. In order to kill the bacterial suspension, formalin was added to both preparations to make a final concentration of 0.25%. The flasks were incubated for 48–72 h at 37°C and then checked for their sterility by usual bacteriological methods. Their innocuity was checked by the usual pathogenicity tests in mice (Bain, 1962).

The two bacterins were prepared from each suspension by mixing three volumes of the suspension and one volume of aluminium hydroxide (Super FOS Danemark). The bacterins, labelled bacterin I (on broth) and bacterin II (on Roux bottles) were stored at refrigerator temperature while their sterility and safety were confirmed by culture and pathogenicity tests in partridges and in mice (Bain, 1962).

Vaccination and challenge exposure

Three groups of 10 birds each were identified by wing band numbers. Groups I and II, which respectively received bacterins I and II, were confined together in one pen, while birds in Group III which served as an unvaccinated control were confined in a second pen. Partridges in Groups I and II were vaccinated twice intramuscularly in the breast at 6 and 8 weeks of age with a 0.5-ml dose containing 9×10^8 organisms of PMM8, each time. The birds in the three groups were challenged intramuscularly 21 days after the second vaccination with $100 \times LD_{50}$ of isolate PMM8. The LD_{50} was predetermined according to the method of Reed and Muench (1938). The LD_{50} was found to be $10^{7.8}$. Birds in each pen were examined post-mortem and their livers, spleens, hearts and bone marrow were cultured.

RESULTS AND DISCUSSION

The two bacterins used were tolerated well by vaccinated partridges. They conferred the same degree of protection in terms of mortality (40%) to a challenge which invariably killed 100% of the controls (Table 1). Table 1 indicates that a similar mortality rate in both groups occurred in the same period. In addition, for both groups, in no case was peracute death observed. This mortality occurred later in comparison with the unvaccinated controls in which it occurred within 24 h.

On necropsy, most dead birds in all groups showed lesions of either general congestion (mostly in the controls) or necrotic foci on the liver and spleen associated with petechiae on the pericardium.

The use of autogenous bacterins is not new and has been applied in the

TABLE 1

Mortality following intramuscular challenge with live *P. multocida* PMM8 after vaccination with autogenous bacterin at 6 and 8 weeks of age

Group	Bacterin preparation	No. dead on day post-challenge				Total dead/ total no. of birds	Percentage mortality
		1-5	6-10	11-15	15-21		
I	Broth medium ^a	0	4 ^c	0	0	4/10	40
II	Solid medium ^b	0	4 ^c	0	0	4/10	40
III	Controls	10 ^c	0	0	0	10/10	100

^aBrain-heart infusion broth (BHB). ^bTrypticase soja agar (TSA). ^cMortality for Groups I and II was recorded between Days 6 and 8 after challenge. Mortality for the controls was recorded within 24 h after challenge.

prevention of fowl cholera in turkeys (Bierer et al., 1961; Michael et al., 1976). In this case, however, the autogenous vaccine was tried experimentally in red partridges before its use on the farm. The present study has again demonstrated the efficacy of using the strains related antigenically to those prevalent on farms. Satisfactory results were obtained, with 60% protection by both bacterin preparations in the presence of a high-challenge dose ($100 \times LD_{50}$). All unvaccinated controls died within 24 h indicating the severe pathogenicity of the challenge inoculum.

It appears that this vaccination programme at 6 and 8 weeks of age using an aluminium hydroxide bacterin would be useful in the field. The first results on the farm are promising and, since this vaccine has been used, no case has been diagnosed on the farm from which the outbreak of fowl cholera was reported (K. Bouzoubaa, unpublished observations, 1987). Finally, since the two bacterins prepared on different media gave a similar protection, production of the vaccine on a broth rather than on a solid medium could be recommended. Large volumes of the vaccine can be produced easily and quickly by this method.

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